AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY 106:1–18 (1998)

Stable Carbon and Oxygen Isotopes in Human Tooth Enamel: Identifying Breastfeeding and Weaning in Prehistory

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KEY WORDS stable isotopes; carbon; oxygen; weaning; diet; breastfeeding; tooth enamel

ABSTRACT This paper investigates the utility of stable carbon and oxygen isotopes in human dental enamel to reveal patterns of breastfeeding and weaning in prehistory. Enamel preserves a record of childhood diet that can be studied in adult skeletons. Comparing different teeth, we used $\delta^{13}C$ to document the introduction of solid foods to infant diets and $\delta^{18}O$ to monitor the decline of breastfeeding. We report enamel carbonate $\delta^{13}C$ and $\delta^{18}O$ of 33 first molars, 35 premolars, and 25 third molars from 35 burials from Kaminaljuyú, an early state in the valley of Guatemala. The skeletons span from Middle Preclassic through Late Postclassic occupations, ca. 700 B.C. to 1500 A.D. Sections of enamel were removed from each tooth spanning from the cusp to the cemento-enamel junction. Stable isotope ratios were measured on CO_2 liberated by reaction of enamel with H_3PO_4 in an automated carbonate system attached to a VG Optima mass spectrometer.

Within a skeleton, teeth developing at older ages are more enriched in ^{13}C and more depleted in ^{18}O than teeth developing at younger ages. Premolars average 0.5% higher in $\delta^{13}C$ than first molars from the same skeleton ($P = 0.0001$), but third molars are not significantly enriched over premolars. The shift from first molars to premolars may be due to the shift to solid foods from lipid-rich milk. After 2 years, when premolars begin to mineralize, the $\delta^{13}C$ in childhood diets did not change systematically. First molars and premolars are similar in $\delta^{18}O$, but third molars average 0.7% lower than first molars ($P = 0.0001$) and 0.5% lower than premolars ($P = 0.0003$). First molar and premolar $\delta^{18}O$ is heavier, because breast milk is more enriched in ^{18}O than is drinking water. Hence, many children continued to nurse during the period of premolar formation. Together, these results indicate that Kaminaljuyú children had begun to eat solid maize foods before the age of 2 years but continued to drink breast milk until much later. Am J Phys Anthropol 106:1–18, 1998.

As human biologists have observed in living populations, early childhood nutrition and morbidity are key determinants of growth, development, and, ultimately, the demographic structure of a population. Cultural beliefs and practices about child rearing as well as access to economic resources shape childhood health experience and, ultimately, affect total population success (Delgado et al., 1982; Dettwyler and Fishman, 1992; Stuart-Macadam and Dettwyler, 1995). Because these issues are critical to reconstructions of past behavior and culture

Received 14 May 1997; Accepted 2 February 1998.

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Contract grant sponsor: Wenner Gren Foundation for Anthropological Research; Contract grant number: 5904;Contract grant sponsor: National Science and Engineering Research Council of Canada.

change, bioarchaeological study of childhood health has become an important research strategy that complements archaeological investigations of prehistoric cultures. Accordingly, bioarchaeologists have developed a battery of research methods that address the nature of childhood health and survival.

Weaning is an important nutritional and health transition for growing children that is determined by cultural ideals as well as biological demands and has implications for child survival. In most cultures, infants are breast fed exclusively at first; at some point, however, they are given solid foods in addition to milk, a diet that is referred to as complementary feeding. Eventually, most nutrients come from solid foods, but children may continue to nurse for several years after solids have become a major part of the diet. This process is best conceived as two separate transitions: 1) the gradual introduction and increased consumption of solid foods and 2) the decline and ultimate cessation of nursing. Herein, we will use the term "weaning" to refer to the end of breast feeding, not to dietary supplementation.

Recently, there has been considerable interest in identifying the age at which prehistoric infants were weaned. Such studies have often focused on indications of infant morbidity through enamel hypoplasias, which result from growth arrest during dental development (Goodman and Rose, 1990). But enamel hypoplasias provide an imperfect reflection of weaning age at best (Judkins and Baker 1996; Katzenberg et al., 1996). It is not possible to separate the effects of nutritional change from morbidity due to infectious disease as causative agents of hypoplasia formation.

Chemical analyses of human remains have given bioarchaeological studies a fruitful way to tease apart the influence of dietary choices and disease processes on prehistoric health (see, e.g., White and Armelagos, 1997; Whittington and Reed, 1997). Work on stable nitrogen isotopes has shown that the shift from milk proteins to proteins obtained from solid foods is registered as a decline in $\delta^{15}N$ of bone collagen (Fogel et al., 1989; Katzenberg et al., 1993, 1996; White and Schwarcz, 1994). Strontium/calcium ratios also show a trophic effect and may shed light on infant feeding practices (Hühne-Osterloh and Grupe, 1989; Sillen and Smith, 1984). These promising methods have been reviewed recently by Stuart-Macadam (1995) and by Katzenberg et al. (1996). They allow insight into the dietary transition and the relative importance of milk versus solid foods to total dietary intake of proteins and calcium, respectively. Thus, they focus more properly on the process of diet supplementation than on weaning, because nursing may continue long after breast milk has ceased to provide most nutrients.

Such paleodietary studies have typically focused on bone as the sample tissue and infer age trends by comparing the chemical signals among skeletons of individuals who had died at varying ages. Although work on $\delta^{15}N$ by Schurr (1997) suggests that peak infant mortality preceded solid supplementation at the Mississippian Angel site, it is possible that the determination of supplementation age and weaning age from deceased infant skeletons may be complicated by the effects of infant feeding practices on mortality, given the nutritional and immunologic benefits of prolonged lactation (Ogra and Losonsky, 1984; Stuart-Macadam and Dettwyler, 1995).

This paper takes a different but complementary approach to examine child nutrition through study of adult skeletons. By focusing on dental enamel of adult skeletons, we gain insight into childhood diets of individuals who survived to adulthood and track changing enamel chemistry during the growth of each individual. We use the stable carbon and oxygen isotopic signal of enamel to monitor the processes of dietary supplementation ($\delta^{13}C$) and changing water sources associated with weaning ($\delta^{18}O$).

Stable carbon isotopic analysis of archaeological bone has now become a common tool for the study of human paleodiets. Stable carbon isotopic signals in plants are determined by the photosynthetic pathway that a plant uses, and these values are passed on to animals that consume the plants. For the Americas, carbon isotopic ratios of bone, $^{13}C\delta$ values[1], have been instrumental in

[1]Isotope ratios of carbon and oxygen are expressed in δ notation as follows: $\delta = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$, where $R = {}^{13}C/{}^{12}C$ for $\delta^{13}C$; and $R = {}^{18}O/{}^{16}O$ for $\delta^{18}O$ and are in units per million (‰).

measuring maize consumption by prehistoric peoples, because maize is the primary New World cultigen that uses the C4 photosynthetic pathway (Schwarcz et al., 1985; van der Merwe and Vogel, 1978; Wright and White, 1996). Experimental work has demonstrated that carbon used to construct bone collagen is derived with some preference from dietary proteins, whereas carbon incorporated into bone mineral (a carbonated hydroxyapatite) represents the bulk average of all dietary macronutrients (Ambrose and Norr, 1993; Tieszen and Fagre, 1993a).

A number of researchers have inferred that maize gruels were fed to Native American infants during the weaning process, before infants were shifted to an adult diet of solid foods. Evidence for this practice has been sought in ethnohistoric records, ceramic technology, circular caries, and stable isotope ratios (Buikstra et al., 1986; Cook and Buikstra, 1979; Katzenberg et al., 1993, 1996). Such a practice should be evident as a change in the $\delta^{13}C$ of enamel forming at the age of initial supplementation.

The ratio of the two stable isotopes of oxygen, ^{18}O and ^{16}O, in body tissues reflects the origin of water imbibed as a liquid and, to a lesser extent, the oxygen obtained from food. Both of these depend on $\delta^{18}O$ of local meteoric precipitation, which varies with latitude because of Rayleigh distillation in the global rainfall cycle. Geochemists have begun to use the oxygen isotopic signal in mammalian bone mineral to reconstruct paleoclimates with considerable success. Because mammals maintain a constant body temperature, $\delta^{18}O$ is recorded in bone mineral at a predictable offset from body water (Longinelli, 1984). For animals or humans living in a single watershed and drinking water of the same isotopic composition, we expect similar isotopic ratios of bone mineral. For mammals, paleoenvironmental studies have documented changes in climate over the long term as well as seasonal fluctuations in climate (Ayliffe and Chivas, 1990; Bryant et al., 1996; Koch et al., 1989; Land et al., 1980; Luz et al., 1990; Stuart-Williams and Schwarcz, 1997). For anthropology, human bone $\delta^{18}O$ holds considerable potential as a tool with which to examine prehistoric migration, because local $\delta^{18}O$ will vary between geographic regions (Stuart-Williams et al., 1996).

Oxygen in body water is enriched in the heavy isotope ^{18}O relative to water imbibed largely due to the preferential expiration of $H_2{}^{16}O$ vapor and admixture of metabolic water containing atmospheric oxygen. The magnitude of this enrichment varies among species due to differences in the contribution of food oxygen and metabolic water to total body oxygen intake (Bryant and Froelich, 1995; Kohn, 1996). Human breast milk is formed from the body water pool and, thus, is heavier in $\delta^{18}O$ than the water imbibed by a lactating mother. Therefore, we might expect the bone mineral of a breast-fed infant to be heavier in $\delta^{18}O$ than that of a bottle-fed infant. If this is true, then $\delta^{18}O$ may prove to be a useful indicator of breast feeding that can be measured in human skeletal remains.

Roberts et al. (1988) have shown that the $\delta^{18}O$ of urine of infants who were breast fed is heavier than that of infants who were bottle fed by an average of 1.85%. Similarly, the average $\delta^{18}O$ of urine from infants fed formula was 2.6% heavier than that of local meteoric water. Either breast milk or formula (mixed from local tap water) provided 95% of water intake by all infants in that study, which was conducted near Cambridge, England. Hence, breast-fed infants show twice the enrichment in ^{18}O relative to meteoric water than children who drink local surface waters. For infants 5–6 weeks of age, Roberts et al. (1988) found that the urine of breast-fed infants was 1.25% more enriched in $\delta^{18}O$ than urine of bottle-fed infants. For infants 11–16 weeks of age, the breast-fed infant urine was 2.69% more enriched than that of bottle-fed infants. Despite the apparent difference between infants of different ages, multiple regression analyses of the data identify the breast-fed/bottle-fed distinction as the only significant variable explaining the $\delta^{18}O$ variation among infants. Roberts et al. (1988) also describe a parallel difference between breast-fed and bottle-fed infants in urine hydrogen isotopic ratios (δD), that could be exploited bioarchaeologically through measurement of δD in bone collagen (see Cormie et al., 1994).

Oxygen occurs in three principal sites in the bone apatite [$Ca_{10}(PO_4)_6(OH)_2$]: in phosphate ions, in hydroxyl ions, and in carbonate ions, which substitute for both the PO_4 and OH (LeGeros and LeGeros, 1984; LeGeros, 1981). To date, most work has focused on $\delta^{18}O$ from PO_4, because it is believed to be more resistant to diagenetic exchange. However, laboratory extraction of oxygen from PO_4 is fairly complex and requires large samples (Stuart-Williams and Schwarcz, 1995). The $\delta^{13}C$ of CO_3 ions is more stable in enamel than in bone apatite due to the closer packing, larger size of enamel crystals, and minimal organic matrix (Lee-Thorp and van der Merwe, 1987, 1991), and this is likely true also for $\delta^{18}O$. Extraction of CO_3 from hydroxyapatite is a simple process, employing a reaction with orthophosphoric acid, which liberates CO_2 from which we obtain both $\delta^{18}O$ and $\delta^{13}C$ data with a single mass spectrometric measurement (McCrae, 1950).

Dental enamel provides the opportunity to obtain information about both the composition of infant diets ($\delta^{13}C$) and the sources of water that they drink ($\delta^{18}O$). Hence, we are able to obtain information regarding two different aspects of the childhood nutritional transition: the introduction of solid foods, which we refer to as "supplementation," and the replacement of mother's milk with alternate sources of water, which we refer to as "weaning." An additional advantage of enamel as the sample tissue is that teeth develop with a known chronology and are not remodeled during subsequent life. Enamel retains childhood isotopic signals into adulthood, preserving a record of dietary and water use changes from childhood that is tied to a known developmental chronology. By comparing age-related changes in $\delta^{13}C$ and $\delta^{18}O$ in enamel carbonate from different teeth in the same skeleton, we can examine the changing proportions of breast milk and solid foods in the diets of prehistoric children as they grew up.

With enamel, we can study childhood nutrition of individuals who survived to adulthood. Previous attempts to examine weaning with $\delta^{15}N$ and Sr/Ca have focused on individuals who died during childhood and who may have been put at greater risk for premature death by differential infant feeding practices. By focusing on enamel of adult skeletons, we can study dietary change over the life of a single individual, instead of among death cohorts.

This paper explores the use of stable isotopic chemistry to examine childhood nutritional transitions at the pre-Hispanic city of Kaminaljuyú in highland Guatemala. We analyzed first molars (M1), premolars (P), and third molars (M3) for a large series of skeletons and used these data to track changes in $\delta^{13}C$ and $\delta^{18}O$ in enamel carbonate with age for each skeleton. The isotopic data show that the shift from mother's milk to solid foods was a very long and gradual nutritional transition for most of the individuals we studied. Kaminaljuyú infants were introduced to solid foods at a young age but continued to drink breast milk until much later.

MATERIALS AND METHODS

Archaeological context

The archaeological site of Kaminaljuyú is situated beneath modern Guatemala City and is now mostly destroyed by urban sprawl. The site was mapped and partially excavated by the Carnegie Institution of Washington (CIW) in the 1930s (Kidder et al., 1946; Shook and Kidder, 1952,) and, in the 1960s, the Pennsylvania State University conducted a broader valley survey and further excavations (Michels, 1979b; Sanders and Michels, 1977; Sanders and Murdy, 1982). Since this time, a number of small rescue projects have been conducted by the Guatemalan Instituto de Antropología y Historia (see, e.g., Lopez, 1992; Popenoe de Hatch, 1993) and the Japanese Museum of Tobacco and Salt (Ohi et al., 1993). Our research grew out of an invitation from Dr. Juan Antonio Valdés to study the human remains recovered by the Proyecto Miraflores, a rescue excavation that was conducted from 1994 to 1996.

Kaminaljuyú was first settled in the Early Preclassic era and grew to a large, socially stratified community by about 400 B.C. Marked differentiation in access to prestige goods during the Late Preclassic period is

indicated by the sumptuous tombs excavated from mound E-III-3 by the CIW (Shook and Kidder, 1952). This early protostate was supported by intensive irrigation agriculture, with canals draining water from Lake Miraflores to a system of irrigated fields on the southern side of the city (Popenoe de Hatch, 1993; Valdés, personal communication). Tectonic shifting changed drainage patterns in the valley near the turn of the millennium, and the lake dried up, but the settlement continued to grow. Ceramics found in Early Classic burials attest to Kaminaljuyu's participation in the Teotihuacanoid horizon style, but they were manufactured locally (Demarest and Foias, 1993). Several elite buildings from this period show talud tablero architecture constructed from adobe (Cheek, 1977; Kidder et al., 1946). Today, archaeologists working at the site and on the Pacific coast of Guatemala do not hold the view that the city was founded by emissaries from Teotihuacan itself, but, instead, they see the city as an indigenous development that participated in a broad interaction sphere with the Pacific coast, Maya lowlands, and Central Mexico locally (Demarest and Foias, 1993). The city reached its peak population during the Late Classic period but was abandoned by Postclassic times, when settlement moved to defensible sites around the valley perimeter (Michels, 1979a; Sanders and Murdy, 1982).

The ancient diet at Kaminaljuyú was similar to that documented for most other highland and lowland Maya sites, based on cultivation of maize, beans, and squash as well as a variety of other fruits and vegetables. Although aquatic fauna, such as turtles and waterfowl, were available locally to the Preclassic community, the Classic Period occupants would have had access only to terrestrial animal meat, such as deer, wild turkey, and domestic dogs. Bone collagen $\delta^{13}C$ for a few adult skeletons averages $-9.8 \pm 1.2\%$ ($n = 6$), intermediate to values obtained on lowland Maya sites (Wright and White, 1996) and those measured by Reed at Postclassic highland Iximché (Whittington and Reed, 1993). However, Kaminaljuyú collagen is isotopically light in $\delta^{15}N$, averaging $7.2 \pm 0.8\%$ ($n = 6$), implying less consumption of higher trophic-level meat than at other Maya sites studied to date (Wright, 1998).

Burial and dental sampling

Teeth were collected from all archaeologically documented skeletal remains. Unfortunately, adequate field documentation could not be found for a large number of remains that have been curated. Ultimately, isotopic data were obtained from 104 teeth from 41 skeletons. Of these, 35 skeletons were sampled with two or more teeth and were used in the present analysis. Six of these were from Proyecto Miraflores remains, 12 were from Pennsylvania State University excavations, one was from San Jorge (Robles, 1994), and 16 were from the CIW excavations (Kidder et al., 1946). These spanned from the Middle Preclassic to the Late Postclassic periods. Most were from Kaminaljuyú proper, but two Late Postclassic skeletons from Belej (Chinautla Viejo) on the north edge of the Valley of Guatemala were also included.

Sample collection focused on the first permanent molar, a premolar, and a third molar. These positions were chosen in order to sample childhood diets during key ages of development with as little developmental overlap as possible between teeth sampled. First molar enamel mineralizes between birth and 3 years of age, premolars mineralize between 2 and 6 years of age, and third molars mineralize during adolescence, between 9 and 12 years (Massler et al., 1941; Moorrees et al., 1963). Where possible, damaged or fragmentary teeth were used instead of sampling intact teeth. Prior to sampling, all anthropometric data were recorded on the teeth, following the guidelines of Buikstra and Ubelaker (1994). These data will be summarized and published in raw form by Wright (1998).

Due to the poor preservation of these remains, which are missing many teeth (many postmortem), it was not possible to obtain the same teeth from all skeletons. Isotopic differences between maxillary and mandibular teeth for first molars and third molars are not expected to be extreme, given the coeval development times for these teeth. With preference, we sampled the third mandibular premolar, but, for many skeletons,

we could select only other premolar positions. Accordingly, we evaluated variability in isotope ratios among tooth-positions and the effect that this might have on isotope ratio trends between teeth.

Analytic methods

All teeth were cleaned ultrasonically with distilled water to remove adhering soil. A section of enamel approximately 2 mm wide that spanned from the cervical margin to the cusp was separated from the tooth crown and cleaned of dentine and surface contamination by using a Foredom diamond bit. Enamel was finely ground by using an agate mortar and pestle to a size of less than 50 µm. Organic components of the enamel were removed by soaking it in a solution of 1.5% sodium hypochlorite over 24 hours, after which the enamel was rinsed with distilled water. Possible diagenetic contaminants were removed by soaking the enamel powder in 1 M acetic acid (buffered with sodium acetate to pH 4.5) for 20 hours. The acid was removed by repeated rinsing in distilled water, and the samples were dried.

We measured the isotope ratios of carbon dioxide evolved from enamel by using the automatic carbonate system of the VG Optima mass spectrometer at McMaster University, which reacts the samples with 100% phosphoric acid at 90°C in a common acid bath. We allowed each 2 mg sample of enamel to react for 12.5 minutes and extended the pump-out time between samples to 12 minutes to ensure completion of the reaction before the following sample was admitted. $\delta^{18}O$ values were corrected for isotopic fractionation between the carbonate and the liberated CO_2, assuming that the fractionation factor was that for calcite ($CaCO_3$) at the same temperature.

For duplicate aliquots on 18 human enamel samples run one after the other, mean deviations were 0.029% for $\delta^{13}C$ and 0.024% for $\delta^{18}O$, comparable to instrument precision, and demonstrated no significant memory effect. For one skeleton (KJ21=33; Palangana Tomb 2, Burial 3), both maxillary and mandibular first molars were prepared and gave comparable results, differing by only 0.208% for $\delta^{13}C$ and only 0.091% for $\delta^{18}O$. Hence, conservatively, we consider differences of 0.5% in $\delta^{13}C$ and 0.2% in $\delta^{18}O$ between teeth to be meaningful.

We calculated yields of CO_2 from each sample by reference to the strength of the major ion beam of the mass spectrometer. We calibrated beam strength for each run using several aliquots of NBS-19 and an internal laboratory calcite standard of known mass. The average difference in yield between repeat aliquots for 14 samples is 0.31%, which we accept as adequately precise. Unfortunately, some samples proved to be too large, and the gas produced was "chopped" by the mass spectrometer, so that yield could not be measured.

We also use Fourier Transform infrared spectrometry (FTIR) to examine hydroxyapatite mineral integrity for a subsample of the teeth. Aliquots of 2 mg of enamel were suspended in potassium bromide pellets, and spectra were collected as described previously (Wright and Schwarcz, 1996). We compared isotopic composition of the enamel to the FTIR crystallinity index (CI; Shemesh, 1990) and the carbonate/phosphate peak ratio (C/P; Wright and Schwarcz, 1996).

RESULTS

Table 1 contains the stable isotopic ratios obtained on all Kaminaljuyú skeletons sampled with two or more teeth.

Enamel diagenesis

For 58 enamel samples examined with FTIR, stable oxygen isotope ratios do not show any systematic correlation with either the apatite CI ($r = -0.18$) or the C/P ($r = 0.04$). Likewise, $\delta^{13}C$ is not related to CI ($r = 0.13$) or C/P ($r = -0.10$). Overall, CI averages 4.8 ± 0.5 and C/P averages 0.07 ± 0.02. For 78 teeth, yields of CO_2 evolved from enamel average $2.4 \pm 0.5\%$ by weight, as calculated from the strength of the major beam. This is similar to the theoretical yield of CO_2 from human enamel, 2.05% by weight, as calculated from the expectation of 2.8% CO_3 by weight (Elliott et al., 1985). $\delta^{13}C$ is not correlated with CO_2 yield ($r = 0.07$), but $\delta^{18}O$ does show a low correlation ($r = 0.35$), which is strongly biased by four samples enriched in ^{18}O that have the highest yields. This small group is also slightly light in

TABLE 1. Stable isotopic data for enamel from skeletons sampled with two or three teeth

Sample code	Burial context	First molar			Premolar			Third molar		
		Pos.[1]	$\delta^{13}C$	$\delta^{18}O$	Pos.	$\delta^{13}C$	$\delta^{18}O$	Pos.	$\delta^{13}C$	$\delta^{18}O$
KJ1	Miraflores B.1	L	−0.79	−5.24	L 4	−0.58	−5.20	U	−1.56	−5.86
KJ2	Miraflores B.2	L	−3.98	−5.09	L 4	−3.73	−5.32	●[2]	●	●
KJ4	Miraflores B.4	L	−4.31	−5.35	U 4	−3.58	−5.30	U	−3.21	−5.81
KJ7	Miraflores B.7	U	−4.27	−5.23	U 4	−4.41	−4.77	L	−3.86	−5.49
KJ10	B-III-3, B.3	U	−3.51	−4.47	U 4	−2.92	−4.78	U	−1.87	−5.89
KJ11	A-VI-6, B.2	U	−1.82	−5.21	U 4	−1.74	−5.11	U	−1.02	−5.30
KJ15	B-V-11, B.1	U	−3.42	−4.77	U 3	−3.59	−4.83	●	●	●
KJ16	B-V-11, B.2	U	−3.61	−4.58	U 3	−2.48	−5.27	U	−2.58	−5.39
KJ18	B-V-6, B.3	L	−4.49	−4.35	L 3	−3.39	−4.86	L	−3.00	−5.36
KJ19	B-V-6, B.4	U	−4.38	−4.46	U 3	−3.55	−5.04	●	●	●
KJ24	Palan. T.II B.2	●	●	●	L 4	−4.29	−6.43	L	−4.25	−6.42
KJ25	Beleh B.1 or 2	L	−4.47	−4.46	L 3	−3.42	−5.47	U	−4.00	−5.04
KJ31	B-V-11, B.5	●	●	●	L 3	−2.43	−5.21	L	−2.51	−5.67
KJ32	B-V-6, B.2	L	−2.29	−5.45	U 3	−2.75	−4.82	U	−2.34	−5.53
KJ33	Palan. T.II B.3	U	−4.55	−4.32	U 3	−2.50	−4.99	L	−1.65	−5.90
KJ34	Test 46-23-012	L	−3.88	−3.82	L 4	−2.21	−3.70	L	−2.25	−5.05
KJ35	B-III-5, B.3	L	−2.23	−4.77	L 4	−3.07	−4.30	L	−3.30	−4.91
KJ36	Beleh B.3	L	−1.09	−5.44	L 4	−0.78	−5.64	L	−0.43	−6.05
KJ40	A-VI-5 D-311	L	−2.58	−4.74	U 4	−1.82	−5.43	●	●	●
KJ41	A-V, skull 3	U	−4.59	−2.25	U 3	−5.38	−2.03	●	●	●
KJ42	A-IV, skel. 2	L	−3.68	−5.14	L 3	−3.36	−5.23	●	●	●
KJ43	A-IV, skel. 1	L	−3.26	−4.15	L 3	−2.31	−4.24	L	−2.18	−5.32
KJ44	A-III, skull 2	U	−4.13	−2.90	U 4	−3.92	−3.12	●	●	●
KJ45	A-I, skel. 3	L	−3.32	−4.19	L 3	−3.26	−3.98	U	−3.89	−4.98
KJ46	A-I, skel. 8	U	−4.45	−1.67	L 3	−3.19	−2.02	U	−3.79	−1.82
KJ47	A-I, skel. 4	L	−3.41	−3.85	L 3	−1.91	−4.51	L	−1.58	−4.21
KJ48	A-II, skel. 2	U	−3.53	−4.57	L 3	−2.79	−4.44	L	−2.29	−4.33
KJ49	A-III, skull 1	L	−6.53	−3.08	U 3	−6.39	−3.33	L	−0.39[3]	−4.93[3]
KJ50	A-I, skel. 9	L	−2.07	−5.02	L 4	−1.64	−5.21	U	−1.63	−5.26
KJ51	A-II, skel. 3	L	−4.06	−4.89	U 3	−3.47	−5.34	●	●	●
KJ52	A-V, skel. 1	L	−2.48	−4.22	L 3	−1.58	−4.50	U	−1.37	−6.76
KJ53	B-IV, skel. 1	L	−2.38	−3.99	L 3	−2.25	−4.02	L	−2.36	−4.61
KJ54	A-III, skull 3	L	−1.46	−2.94	U 3	−1.59	−3.06	U	−3.50	−3.40
KJ55	B-IV, skel. 2	L	−6.86	−2.36	L 3	−5.94	−2.69	●	●	●
KJ56	A-IV, skel. 3	L	−2.54	−3.50	L 3	−1.45	−3.43	●	●	●

[1] Tooth position: U, maxillary; L, mandibular; 3, third premolar; 4, fourth premolar.
[2] This tooth was not available; therefore, it was not sampled.
[3] Suspected diagenesis or contamination, excluded from statistical comparisons.

$\delta^{13}C$, although it is not light enough to affect the lack of correlation. Although diagenesis cannot be ruled out, it is possible that the difference is a cultural one, owing to a distinction in diet, water sources, or geographic origin. Beam strengths obtained on modern human enamel are comparable to samples of Kaminaljuyú enamel for a given mass. Although these findings do not provide firm evidence that diagenetic exchange has not taken place, they do suggest that the composition of the enamel has not been changed markedly since its interment.

Isotopic patterning among teeth

Figure 1 illustrates the isotopic composition of first molars and third molars from Kaminaljuyú for all skeletons sampled with two or more teeth. Although there is a broad variation among teeth in both $\delta^{18}O$ and $\delta^{13}C$, it is evident that first molars tend toward the top left of the cluster, whereas third molars tend toward the bottom right of the cluster. Reviewing Table 1, we see that the first molar tends to be more enriched in ^{18}O and somewhat depleted in ^{13}C than the premolar and the third molar. However, the broad variation among skeletons in absolute values of $\delta^{13}C$ and $\delta^{18}O$ makes this difficult to appreciate from Figure 1. We suspect that social variability in diet and chronological dietary change among the skeletons sampled account for the broad spread in $\delta^{13}C$. The variation in $\delta^{18}O$ could be due to changing environmental factors, such as the disappearance of Lake Miraflores, as well as possible differential access among social groups to water resources, and foreign immi-

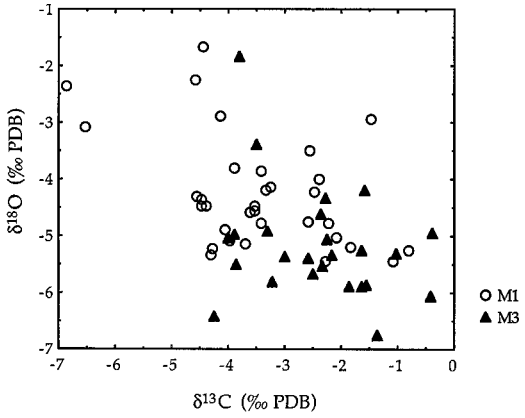

Fig. 1. Stable isotopic ratios of first molar and third molar enamel carbonate from Kaminaljuyú skeletons for which more than two teeth were sampled.

gration. We will examine this variability in $\delta^{13}C$ and $\delta^{18}O$ in detail elsewhere.

To examine possible shifts in isotopic composition between teeth from a single skeleton, instead, we focused on the difference in $\delta^{13}C$ and $\delta^{18}O$ between pairs of teeth, always subtracting the isotopic composition of the later developing tooth from the composition of the tooth that developed earlier. We designate ΔC and ΔO as follows:

$$\Delta C = \delta^{13}C_{early} - \delta^{13}C_{late}.$$

$$\text{Thus, } \Delta C_{M1\text{-}P} = \delta^{13}C_{M1} - \delta^{13}C_{P},$$

$$\text{and } \Delta C_{M1\text{-}M3} = \delta^{13}C_{M1} - \delta^{13}C_{M3},$$

$$\text{and } \Delta C_{P\text{-}M3} = \delta^{13}C_{P} - \delta^{13}C_{M3},$$

and, similarly, for ΔO. A negative ΔC or ΔO value means that the later developing tooth is more enriched in the heavy isotope than the early developing tooth, whereas a positive value means that the later developing tooth is isotopically lighter than the early developing tooth.

Tooth position comparability

Because of the fragmentary nature of the remains, we were not able to sample the same teeth from each skeleton but had to be opportunistic. Our sample contains maxillary and mandibular first and third molars and all four premolars. We would not expect a systematic difference between maxillary and mandibular molars for each position, because these teeth develop at the same time. This could be a bigger problem for the premolars, because mandibular third premolar enamel forms from 1 to 6 years, whereas the remaining premolars form from 2 to 6 years or from 2 to 7 years. Hence, skeletons sampled with a lower third premolar are biased toward diets at a slightly younger age.

Table 2 summarizes the results of two-factor analyses of variance (ANOVAs) that tested for differences in ΔO and ΔC that might be attributable to tooth position. For each of our pairs of ΔO comparisons, two-way ANOVAs indicate no statistical differences in $\delta^{18}O$ between tooth positions. Likewise for $\delta^{13}C$, most ΔC comparisons are not significant; however, for $\Delta C_{P\text{-}M3}$, the F value is statistically significant. This result is influenced strongly by one sample, KJ49, which shows a markedly ^{13}C-enriched M3, so much so that we suspect that it was altered diagenetically or was contaminated in the laboratory. If we exclude this sample, then only the one-way ANOVA on M3 remains statistically significant. Given the lack of significance of the remaining ΔC tests, the small number of individuals in each cell of the ANOVA, and the likelihood of obtaining a type I error, we cautiously conclude that tooth position does not have a large effect on isotopic variability among the data. The segment of enamel removed from each tooth spans the full period of amelogenesis, but its greatest depth corresponds to the middle of this time, which accounts for most of the isotopic signal. The broad overlap between teeth in mineralization times, coupled with individual variation in development, overshadows any such differences between teeth. Therefore, we consider isotopic composition of teeth grouped only as first molars (M1), premolars (P), and third molars (M3).

Age trends in $\delta^{13}C$ and $\delta^{18}O$

Figure 2a illustrates mean ΔC for each tooth-pair comparison. Overall, the $\Delta C_{M1\text{-}P}$ is -0.5%, indicating that premolars average 0.5% heavier than first molars in $\delta^{13}C$. Likewise, first molars are 0.6% heavier than third molars. By contrast, premolars are not

TABLE 2. Analyses of variance examining the effect of tooth position on ΔC and ΔO

ANOVA	Degrees of freedom	ΔC		ΔO	
		F	*P*	F	*P*
ΔM1-P (all positions)					
M1 (A)	0	●[1]		●	
P (B)	2	1.548	0.231	0.026	0.974
AB	2	1.253	0.302	1.185	0.322
ΔM1-P (excluding mandibular P4)					
M1 (A)	1	0.064	0.802	0.099	0.756
P (B)	2	1.751	0.198	0.024	0.977
AB	2	1.420	0.264	1.244	0.309
ΔM1–M3 (all positions)					
M1 (A)	1	0.657	0.428	0.480	0.497
M3 (B)	1	2.102	0.163	0.012	0.914
AB	1	0.567	0.461	0.139	0.713
ΔP-M3 (all positions)					
P (A)	3	3.063	0.056	0.297	0.827
M3 (B)	1	6.840	0.018	0.553	0.467
AB	3	3.804	0.030	0.785	0.519
ΔP-M3 (excluding KJ49)					
P (A)	3	1.876	0.174	0.080	0.970
M3 (B)	1	4.833	0.043	0.183	0.674
AB	3	1.038	0.402	0.341	0.796

[1] Could not be calculated due to small sample size. ANOVA, analysis of variance.

significantly different in $\delta^{13}C$ from third molars, as shown by the average $\Delta C_{P\text{-}M3}$ of zero. This indicates that, on average, enamel $\delta^{13}C$ becomes heavier with increasing age during childhood and that most of this shift occurs between the first molar and the premolar, or by 3 years of age. After the age of 3 years, enamel $\delta^{13}C$ and, thus, diet do not change systematically with increasing age.

Similarly, Figure 2b illustrates trends in ΔO between teeth. By contrast with ΔC, ΔO values are positive, indicating a trend toward lighter $\delta^{18}O$ with increasing age. On average, there is a very small difference between first molars and premolars, with a mean $\Delta O_{M1\text{-}P}$ near +0.2%. However, third molars average 0.7% lighter than first molars and 0.6% lighter than premolars, as indicated by the ΔO values. This indicates that the shift toward lighter $\delta^{18}O$ with age occurs in large part between the time of mineralization of the premolar and the third molar, that is, after 6 years and before 9 years of age.

Table 3 gives the results of paired t-tests on $\delta^{13}C$ and $\delta^{18}O$ for comparisons between teeth from the same skeleton that serve to test the statistical significance of these ΔC and ΔO trends. The mean differences in $\delta^{13}C$ and $\delta^{18}O$ between paired teeth are equal to mean ΔC and ΔO for the data as a whole. For $\delta^{13}C$, the t-tests confirm that first molars are significantly different from both premolars and third molars, whereas premolars and third molars are not significantly different. For $\delta^{18}O$, all three tooth-position comparisons are statistically significant. However, the magnitude of the $\Delta O_{M1\text{-}P}$ difference is small and is at the limit of measurable difference.

Although the age trends in both ΔC and ΔO are highly significant, it is evident from the error bars in Figure 2 that there is a large degree of variability in ΔC and ΔO among burials. Figure 3 illustrates this variability for each tooth-pair comparison by plotting ΔC against ΔO. Figure 3a shows that changes in $\delta^{13}C$ and $\delta^{18}O$ between the first molar and the premolar are highly correlated ($r = -0.66$). Note that the data cluster closely together, in contrast to Figure 3b,c. Many skeletons fall close to the origin, indicating no substantial change in either isotope ratio, whereas others span up to a 1% change in $\delta^{18}O$ and a 2% change in $\delta^{13}C$. Four skeletons lie in the bottom right quadrant of the graph, indicating that the premolar was more enriched in both ^{13}C and ^{18}O than the first molar, but they lie along the same trend.

Figure 3b illustrates ΔC and ΔO for the comparison between first molars and third molars. Unlike Figure 3a, few skeletons fall close to the origin. ΔC and ΔO show a lower

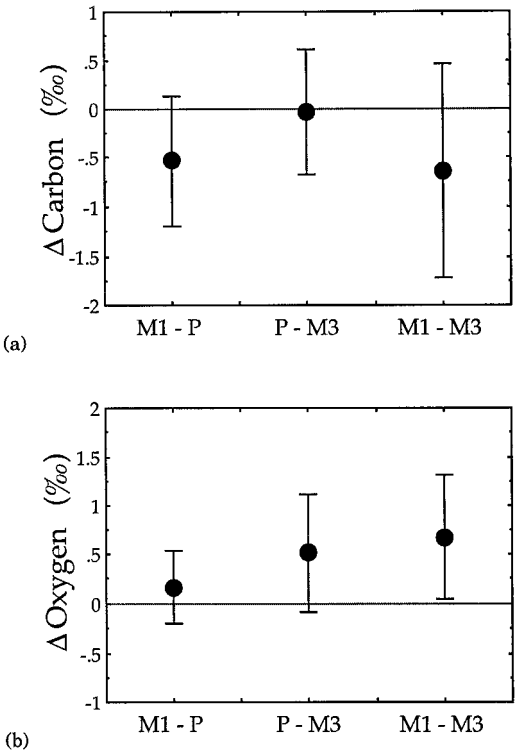

Fig. 2. Mean difference in stable isotopic composition between pairs of teeth. **a:** Stable carbon isotopic composition. **b:** Stable oxygen isotopic composition.

correlation ($r = -0.40$) than in the previous comparison. Only one skeleton shows a negative $\Delta O_{M1\text{-}M3}$ value, and it is a small difference that might be due to measurement error. Most skeletons show negative values for $\Delta C_{M1\text{-}M3}$, although four skeletons show ^{13}C depletion with age. Figure 3c shows that ΔC and ΔO vary independently between premolars and third molars: They are not correlated ($r = -0.18$). Similar to $\Delta O_{M1\text{-}M3}$, $\Delta O_{P\text{-}M3}$ is positive for almost all samples, but $\Delta C_{P\text{-}M3}$ ranges from -1 to $+2\%$.

Figure 4 illustrates a significant relationship between carbon isotope change in teeth and the composition of first-molar enamel. The $\delta^{13}C$ of first molars is correlated positively with $\Delta C_{M1\text{-}M3}$ ($r = 0.60$; $n = 22$) and shows a weaker correlation with $\Delta C_{M1\text{-}P}$ ($r = 0.35$; $n = 32$). Similar correlations are not found for $\delta^{18}O$ and ΔO or between $\delta^{13}C_P$ and $\delta^{13}C_{M3}$ with ΔC.

DISCUSSION

Age trends in $\delta^{13}C$: Introduction of solid foods

By comparing $\delta^{13}C$ among first molars, premolars, and third molars, we observe a general enrichment with increasing age, most of which change occurs between the first molar and the premolar. Because carbonate $\delta^{13}C$ in apatite is known to reflect whole-diet $\delta^{13}C$ (Ambrose and Norr, 1993; Tieszen and Fagre, 1993a), we might interpret this trend as a shift in nutrient sources. We assume that Kaminaljuyú infants were breast fed initially, and, at some time, solid foods were introduced to the diet, ultimately providing all nutrients. From the enriched $\delta^{13}C$ of premolars, we might infer that children 2 to 6 years of age were eating more maize foods than they had eaten as infants. This might be expected if weanling diets consisted of maize gruel and, thus, were heavier isotopically than milk, which, in turn, reflects maternal diet.

Alternatively, isotopic differences between maternal diet and milk may also explain this trend independent of any special weanling food. For Holstein cows, milk $\delta^{13}C$ equilibrates rapidly to a dietary change. Milk of cows in late lactation on a corn diet was found to be 2.2% lighter than the cows' diet (Boutton et al., 1988), but the magnitude of this difference was much less (only 0.2%) for cows that were fed alfalfa. Boutton et al. (1988) favor a fractionation process during milk synthesis to account for this difference. By contrast, Mitchell et al. (1993) describe ^{13}C enrichment of milk over diet for pigs in early lactation on C3 diets and little fractionation between diet and milk for pigs on C4 diets. These differences may be due to greater

TABLE 3. Results of paired Student's t-tests on $\delta^{13}C$ and $\delta^{18}O$ between teeth

Tooth pair	Mean difference	Degrees of freedom	Student's t-test	*P* (two-tail)
$\delta^{13}C$				
M1-P	−0.529	32	−4.59	0.0001
M1–M3	−0.636	21	−2.74	0.0122
P-M3	−0.027	23	−0.20	0.8408
$\delta^{18}O$				
M1-P	0.167	32	2.59	0.0143
M1–M3	0.680	21	5.10	0.0001
P-M3	0.522	23	4.28	0.0003

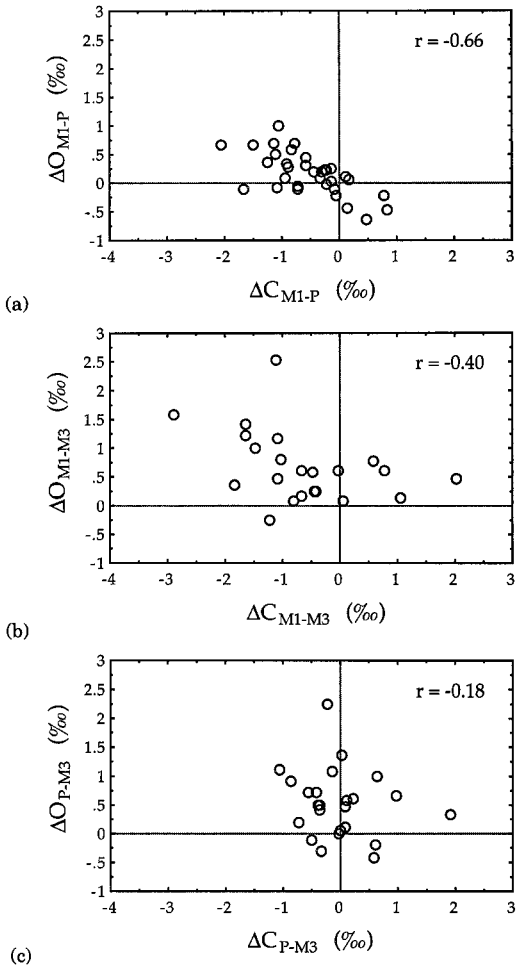

Fig. 3. Correlation between change in $\delta^{13}C$ and $\delta^{18}O$ between pairs of teeth from first molars to premolars (**a**), from first molars to third molars (**b**), and from premolars to third molars (**c**).

use of body stores in milk production during early lactation by the pigs or to insufficient diet equilibration in Mitchell et al.'s experimental protocol. We were unable to find any comparable isotopic data for human milk.

The direction and magnitude of fractionation in milk synthesis could also be due to the sources of lipids in breast milk. Lipids constitute 33% of dry matter in human milk and provide 54% of energy obtained from breast milk (Oftedal 1984). Milk fats are obtained partly from dietary triglycerides in blood plasma, but they are also synthesized from blood glucose in the breast (Williamson et al., 1984), during which process we anticipate fractionation to a ^{13}C-depleted product (DeNiro and Epstein, 1977). Overall dietary lipids tend to be lighter isotopically than other macronutrients, even for monoisotopic diets. For instance, within a single kernel of maize, the lipids are 4–6% lighter than the carbohydrates (Tieszen and Fagre, 1993b). Although maize would contribute some lipids to prehistoric Kaminaljuyú diets, lipids would have come primarily from C3 sources, such as fauna, cacao, avocados, and pumpkin seeds. By contrast, carbohydrates would have come predominantly from C4 maize. Therefore, for Kaminaljuyú, we expect milk fat to be isotopically lighter than maternal diet as a whole, although we cannot estimate the magnitude of this difference without experimental work. Certainly, data on the isotopic composition of maternal diets and milk would strengthen our interpretation, but their collection is beyond the scope of this paper.

Accordingly, we expect a Kaminaljuyú child consuming only breast milk to have apatite $\delta^{13}C$ that is slightly lighter than that of a child eating the same diet as its mother. Because lipids in milk provide a great deal of milk carbon, the observed difference between first-molar and premolar $\delta^{13}C$ might

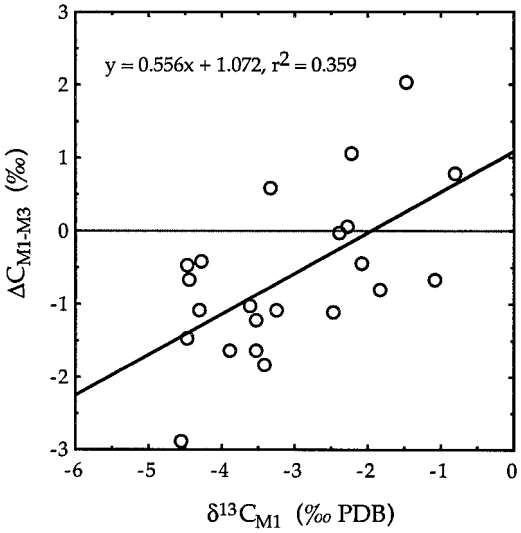

Fig. 4. Relationship between $\delta^{13}C$ of first molars and ΔC_{M1-M3}.

reflect only a decline in milk consumption or in the fat content of milk. Lipid content in milk varies with the intensity and frequency of nursing (Quandt, 1984; Woolridge, 1995), so mothers who nurse only briefly or at long intervals will produce milk that is lower in fat than mothers who nurse more frequently for longer durations.

If human milk is depleted systematically in ^{13}C, then, once a child begins to eat solid food, we expect enamel $\delta^{13}C$ to become heavier, assuming that infants eat solid diets identical to those of their mothers. If children are fed a maize weanling gruel, then we would expect even greater enrichment in $\delta^{13}C$ because of both the ^{13}C enrichment of maize and the low lipid content of maize-based diets. On average, children require additional nutrients by 6 months of age, or growth may falter. Human cultures vary dramatically in the age at which solid foods are introduced. Among rural Maya communities studied by the Institute of Nutrition of Central America and Panama in Guatemala, faltering may begin by 3 months of age in exclusively breast-fed infants (Rivera and Ruel, 1997; see also Butte et al., 1992). However, other concerns, such as the contamination of alternate foods and cultural perceptions, can lead mothers to delay supplementation of the milk diet to 1 year of age or later (Dettwyler and Fishman, 1992). In Guatemala today, the mean duration of breast feeding in indigenous communities is 24 months, but data on the introduction of solid foods are more difficult to obtain. In eastern Guatemala, Ladinos consider solid food inappropriate for young children and begin to feed them small quantities of softened tortillas, watery maize gruel (called *atole*), and black-bean broth after 6 months of age while continuing breast feeding for an average of 15 months (Izurieta and Larson-Brown, 1995).

We assume that Kaminaljuyú children had begun to supplement breast milk with solid foods by at least 1 year of age. The period of first molar development spans birth to 3 years and includes this period of dietary change. We interpret the $\delta^{13}C$ of first molars as a net result first of $\delta^{13}C$ depletion from breast milk, followed by some enrichment from solid food as it is introduced. Therefore, for those supplemented early, the observed ΔC is less than we would expect if we could measure the $\delta^{13}C$ of presupplemented enamel more precisely. Children for whom breast milk remained a major source of dietary nutrients beyond 2 years have smaller $\Delta C_{M1\text{-}P}$ values ($<0.5\%$). The larger $\Delta C_{M1\text{-}P}$ ($\geq 0.5\%$) observed for most individuals implies that many Kaminaljuyú children had been introduced to a diet containing substantial solid foods by 2 years of age, when the premolars began to mineralize. After 2 years, the contribution of milk to carbon intake was less than that of solid food. Although it is not necessary to infer the use of maize gruel (*atole*) to explain this trend, we cannot rule out this practice.

After 2 years of age, childhood diet overall at Kaminaljuyú appears to have been stable in $\delta^{13}C$, as indicated by the lack of systematic differences between premolars and third molars and by the fact that the difference between first and third molars is comparable to that between first molars and premolars, although it is more variable. However, Figure 3c demonstrates that many children's diets did change substantially (up to 2%) between the ages 6 years and 9 years. Such changes vary widely and are not consistent among skeletons. For those infants who showed a decline in $\delta^{13}C$, we might suspect that the premolar enrichment was due partly to weanling *atole* consumption. Further enrichment implies greater reliance on maize, perhaps due to individual preference or variation in family status and economics.

The correlation shown in Figure 4 indicates that carbon isotopic change between teeth is greater for children who had lower first-molar $\delta^{13}C$. These lower $\delta^{13}C_{M1}$ values indicate either that these children did not begin to supplement breast milk with solid food until quite late, perhaps after the first molar had completed mineralization, or that their mothers consumed far less maize and weaned their infants onto a diet containing more maize than they ate themselves. Those individuals with heavier $\delta^{13}C_{M1}$ may have been supplemented earlier or may have had mothers who ate more maize themselves.

At first glance, the observed trend toward increasing $\delta^{13}C$ with older age among Kaminaljuyú children stands in contrast to that

observed in other paleodietary studies. Katzenberg et al. (1993) found that the bone collagen of Iroquoian children who had died before 5 years of age was more enriched in ^{13}C than the collagen of adults from the same site. They suggest that this enrichment is due either to a trophic level effect of ^{13}C enrichment from breast milk proteins or to the practice of feeding infants a maize gruel, as recorded ethnohistorically (Katzenberg et al., 1993, 1996). Similar trends in $\delta^{13}C$ have been described by Tuross and Fogel (1994) among Plains skeletons. These studies document $\delta^{13}C$ enrichment of collagen even for toddlers under 2 years of age. Because this is accompanied by enrichment in $\delta^{15}N$, which is best explained as a trophic effect of milk consumption, Katzenberg and colleagues (1993, 1996) seem to favor a trophic effect to explain the ^{13}C-enriched collagen of children compared with adults. Katzenberg et al.'s (1993) collagen data do not show a systematic difference between infant ($<$1 year) and child ($>$1 year) collagen $\delta^{13}C$.

Unfortunately, poor collagen preservation at Kaminaljuyú does not permit a comparative study of bone collagen $\delta^{13}C$ with age. Among the lowland Maya, subadult bone collagen that is more ^{13}C-enriched than adult bone collagen has been documented at Topoxté (Acevedo et al., 1997). An analogous shift in enamel is not evident between premolars and third molars for Kaminaljuyú. However, age changes studied in bone do not follow each individual through life but compare those deceased as infants to surviving adults. Were the ^{13}C-enrichment due to different weanling diets, these might also be linked to early mortality. Moreover, the bone collagen studies have compared subadult diets with adult diets, not infant diets with child diets, which are the focus of this enamel study.

The apparently conflicting trends between the collagen of deceased infants and the enamel of surviving children might also be a consequence of the different carbon pools of macronutrients used in the synthesis of these two tissues. Experimental data and theoretical considerations indicate that collagen is synthesized with some bias from dietary proteins and that dietary lipids are partially barred from contributing to collagen. By contrast, lipids and carbohydrates contribute more fully to apatite carbon (Ambrose and Norr, 1993; Schwarcz, 1999). Hence, the more enriched $\delta^{13}C$ of infant collagen compared with enamel carbonate could reflect preferential routing of carbon atoms from milk protein, which we expect to be more ^{13}C-enriched than those of milk fat. By contrast, if milk fat is isotopically lighter than the diet consumed by a lactating mother, as we suspect, then apatite will become more ^{13}C-enriched as the main source of dietary carbon used to synthesize enamel switches from milk fat to carbohydrates in solid foods, even if infant diet is identical to maternal diet.

Certainly, if infants were fed a special maize-based weaning food, like *atole*, then their enamel would be enriched even further in ^{13}C. Also, we would expect that a special gruel might be used only before the deciduous dentition had erupted; whereas, after 2 years of age, we would expect children to experiment with a more diverse, solid diet. The few individuals who showed a positive $\Delta C_{M1\text{-}P}$ in Figure 3a could be examples of infants who were fed a ^{13}C-enriched atole diet at a very young age, in which case, their lighter premolar values would be explained by greater dietary diversification after 2 years of age. However, for the majority of skeletons (who showed positive values of $\Delta C_{M1\text{-}P}$), it is not necessary to posit a maize-based weanling food to account for the observed age-related trends in either enamel or bone.

Age trends in $\delta^{18}O$: Changing drinking patterns

By contrast with ΔC, first molars and premolars differ little in $\delta^{18}O$. ΔO is close to zero, indicating only a slight change in the isotopic composition of water imbibed by children between birth and 6 years of age. Instead, a decline of 0.6% in $\delta^{18}O$ between premolars and third molars is evident. Both premolars and first molars are significantly heavier than third molars. The consistency of $\Delta O_{M1\text{-}P}$ and $\Delta O_{M1\text{-}M3}$ hints that water sources remained essentially stable between birth and 6 years of age and then shifted to an isotopically lighter water after 6 years of

age. The best explanation for this difference is that children less than 6 years of age drank isotopically enriched water compared with the water they would drink at older ages. The most likely source of this ^{18}O-enriched water is maternal breast milk. Although the net $\delta^{18}O$ change is consistent, there is substantial variability among skeletons in the timing of this shift. A few skeletons show a decline in $\delta^{18}O$ of 0.5% between the first molar and the premolar, indicating substantial consumption of water soon after 2 years of age. However, most skeletons show shifts greater than 0.5% between the premolar and the third molar (Fig. 3b,c), indicating that, for many children, breast milk continued to provide a substantial quantity of water imbibed throughout the period of premolar mineralization, perhaps up to the age of 6 years.

The magnitude of this $\delta^{18}O$ shift is only half to one-third of the difference observed in urine $\delta^{18}O$ between breast-fed and bottle-fed English babies (Roberts et al., 1988). Yet, several Kaminaljuyú skeletons do show larger shifts on the order of those measured by Roberts et al. (1988). We hypothesize that the variability observed in ΔO may be due to differences in breastfeeding frequency versus water supplementation among segments of the Kaminaljuyú population; this social variability will be examined in greater detail elsewhere. However, we note that the enamel sampling procedure we have used pools together enamel produced over the full mineralization span of each tooth. Because of thicker enamel deposition in the middle of the crown, our isotopic measurements are biased toward diet and water sources during the midpoint of enamel mineralization for a given tooth. Because crown mineralization begins at the cusp and progresses cervically, $\delta^{18}O$ at the tooth cusp and at the cemento-enamel junction (for premolars and some first molars) are likely more enriched and depleted, respectively, than the "total" values reported here. Hence, the true shift in $\delta^{18}O$ over childhood is probably slightly greater than our measured values.

For children at Kaminaljuyú, alternate sources of water would be rain water or stream water, which would be used in cooking and preparing beverages, in addition to water in fruits, other plants, and animal foods. If it was stored in ceramic pots for extended periods, rain water could become enriched by evaporation. Likewise, water in fruits might be enriched over soil water taken up by tree roots. ^{18}O enrichment of stored water should affect adults and children equally, and is unlikely to account for our findings. If infants were fed disproportionate amounts of fruit, then this might contribute to the observed trend. However, the volume of water imbibed from breast milk or other water-based beverages is likely to have been much greater than that obtained from fruits.

More significant might be ^{18}O enrichment from extended boiling of beverages (Fricke et al., 1996). *Atole*, a common breakfast drink of the Maya, is prepared by boiling ground maize with water (Benedict and Steggerda, 1937) and could have been fed disproportionately to young children, as mentioned previously. However, if ^{18}O enrichment of first molar and premolar enamel was due to ^{18}O-enriched *atole*, then we would expect a *decline* in $\delta^{13}C$ to parallel the observed decline in $\delta^{18}O$ as older children begin to eat more diversified diets. Instead, we observe a *rise* in $\delta^{13}C$ at an earlier age and *no* $\delta^{13}C$ change concomitant with the $\delta^{18}O$ shift. The introduction of solid foods appears to have affected the $\delta^{13}C$ of enamel independently from $\delta^{18}O$ in terms of both the timing and the direction of isotopic shifts. Moreover, Guatemalan Ladinos dilute *atole* substantially before feeding it to infants (Izurieta and Larson-Brown, 1995). Dilution with fresh, cool water (to avoid a burn) could lower the $\delta^{18}O$ of *atole* substantially.

Systematic differences between teeth in the $\delta^{18}O$ of enamel phosphate have also been described for horses by Bryant et al. (1996) and for sheep by Fricke and O'Neil (1996). They also came to the conclusion that this variation was due to ^{18}O-enriched milk. White (1997) has also observed ^{18}O enrichment of bone phosphate in some infants' skeletons from Teotihuacan.

We have also considered the possibility that the changes in $\delta^{18}O$ might be related to the higher metabolic rates of young children, to changing activity patterns with age,

or to more rapid water use with smaller body size, because body size and metabolic rate affect the magnitude of isotopic fluxes and fractionation (Bryant and Froelich, 1995; Kohn, 1996). If changes in $\delta^{18}O$ were a factor of age-related metabolic differences, activity patterns, or growth trends, then we would expect them to be quite consistent among skeletons. However, we have documented substantial variation in the timing of the shifts in $\delta^{18}O$ composition within the Kaminaljuyú population. This cannot be explained easily by differences in metabolic rate or body size, because we sampled only skeletons of individuals who survived to adulthood. Those with impaired growth presumably were removed from the population as infants or toddlers and are not among our sample. Moreover, Roberts et al. (1988) did not find infant age, growth rate, or body weight to be significant predictors of urine $\delta^{18}O$ in their comparison of breast-fed and bottle-fed infants. Cultural differences in the timing of weaning are more likely to account for the observed variation in timing of $\delta^{18}O$ shifts.

CONCLUSIONS

In conjunction with stable carbon isotopes, oxygen isotopes appear to shed light on the duration of breast feeding in archaeological skeletons. The nonsynchronous timing of shifts in $\delta^{13}C$ and $\delta^{18}O$ demonstrates that Kaminaljuyú mothers practiced extended complementary feeding. The isotopic composition of Kaminaljuyú enamel indicates that many children continued to drink ^{18}O-enriched milk between the ages of 2 years and 6 years, when the premolars were mineralizing. Although solid foods had been introduced early, before the premolars began mineralizing, breast milk appears to have provided a significant proportion of liquids imbibed by many Kaminaljuyú children up to the age of 5 or 6 years. This age corresponds well with the ethnohistoric data for colonial Yucatán, where Bishop Diego de Landa observed that Maya infants were "weaned" at 4 years of age (Tozzer, 1941). This age also matches Dettwyler's (1995) calculation that the "natural" age of weaning for humans falls between 2.5 years and 7.0 years, which she estimates by reference to weaning age and life history variables among nonhuman primates.

However, it is important to emphasize that the $\delta^{18}O$ shifts track changes in the relative importance of milk versus rain water to total liquid intake. $\delta^{18}O$ does not permit us to identify a final nursing event. Moreover, we suggest that weaning (cessation of breast feeding) occurred for many infants around 5 or 6 years of age, on the basis of the bulk composition of premolar enamel, which is probably biased toward ages 3–5 years. By contrast, $\delta^{13}C$ changes track the supplementation of the milk diet with solid foods, which occurred several years before weaning. The use of a special maize weanling food is not demonstrated conclusively by the observed $\delta^{13}C$ trends between teeth, but it cannot be ruled out either. It should be possible to obtain a more precise chronology of $\delta^{13}C$ and $\delta^{18}O$ change through microsampling of enamel from cusp to cervix of the crown, a strategy that we are currently attempting. This would permit a more detailed understanding of infant feeding practices.

We have also documented considerable variability in the timing of both $\delta^{13}C$ and $\delta^{18}O$ shifts among the ancient inhabitants of Kaminaljuyú. To some extent, this variation in the duration of lactation may simply reflect idiosyncratic variability in child growth, nutritional demands, and maternal competence. However, dietary shifts over the long occupation of the site and discrepancies among social groups may also pertain. The duration of breast feeding is culturally determined and often varies among social status groups within a culture, in accord with disparity in economic success (Dow, 1977; Martorell and O'Gara, 1985; Pérez-Escamilla, 1993). We will explore the social correlates of $\delta^{13}C$ and $\delta^{18}O$ variation among social groups at Kaminaljuyú in a forthcoming paper.

Although the results presented here would benefit from a more detailed chronology of isotopic change with age, the observed patterns demonstrate the potential of stable carbon and oxygen isotopes in dental enamel to shed light on infant feeding practices in prehistory. Significantly, $\delta^{13}C$ and $\delta^{18}O$ tie food and water consumption to specific devel-

opmental ages through a focus on dental enamel as the sample tissue and permit a "diachronic" study within individuals. Moreover, when they are considered together, the two isotopes distinguish between the introduction of solid foods to infant diets and the duration of lactation, thereby permitting a more detailed analysis of infant feeding practices in the past. Complementing $\delta^{15}N$ and Sr/Ca studies, we hope that this approach will prove to be a successful strategy with which to explore the biocultural implications of variability in breast feeding in ancient cultures.

ACKNOWLEDGMENTS

This research was funded by a grant from the Wenner Gren Foundation for Anthropological Research (grant 5904) to L.E.W. and also benefitted from laboratory support from the National Science and Engineering Research Council of Canada to H.P.S. The laboratory research was conducted during L.E.W.'s tenure of a Social Science and Humanities Research Council of Canada postdoctoral fellowship at McMaster University. In Guatemala, we thank Dr. Juan Antonio Valdés for encouraging us to undertake this research. Lic. Erik Ponciano generously granted access to the skeletal remains under the care of the Instituto de Antropología e Historia and gave permission for the dental sampling. Licda. Dora Guerra de Gonzalez and Dr. Marion Popenoe de Hatch kindly allowed us to excavate and sample the burial from San Jorge in the Museo Nacional de Arqueología y Etnología. Jose Genovés, Mario Vasques, and Benito Burgos enthusiastically helped with the osteological documentation. At the Peabody Museum of Archaeology and Ethnohistory, Dr. Larry Flynn, Gloria Greis, and Genevieve Fisher were helpful in setting up our research visit and expedited our sampling request for the CIW materials. At McMaster University, we thank Martin Knyf and Hilary Stuart-Williams for their kind technical advice and Inés Guerrero, who helped with sample preparation and conducted most of the infrared spectrometry. Kathy Dettwyler offered some critical comments on breast feeding that helped to improve this paper. We thank four anonymous reviewers for their helpful comments, especially the one who pointed out the correlation shown in Figure 4.

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